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Mechanisms of *Artemisia scoparia*'s Anti-Inflammatory Activity in Cultured Adipocytes, Macrophages, and Pancreatic β -Cells

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For chronic weight management in adults with a BMI of ≥ 30 kg/m² (obesity), or ≥ 27 kg/m² (overweight) in the presence of a weight-related comorbidity, as an adjunct to a reduced calorie diet and increased physical activity.



ONCE - WEEKLY

wegovy™

semaglutide injection 2.4 mg

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Indications and Usage

Wegovy™ (semaglutide) injection 2.4 mg is indicated as an adjunct to a reduced calorie diet and increased physical activity for chronic weight management in adults with an initial body mass index (BMI) of ≥ 30 kg/m² (obesity) or ≥ 27 kg/m² (overweight) in the presence of at least one weight-related comorbid condition (e.g., hypertension, type 2 diabetes mellitus, or dyslipidemia).

Limitations of Use

- Wegovy™ contains semaglutide and should not be coadministered with other semaglutide-containing products or with any GLP-1 receptor agonist.
- The safety and effectiveness of Wegovy™ in combination with other products intended for weight loss, including prescription drugs, over-the-counter drugs, and herbal preparations, have not been established.
- Wegovy™ has not been studied in patients with a history of pancreatitis.

Important Safety Information

WARNING: RISK OF THYROID C-CELL TUMORS

- In rodents, semaglutide causes dose-dependent and treatment-duration-dependent thyroid C-cell tumors at clinically relevant exposures. It is unknown whether Wegovy™ causes thyroid C-cell tumors, including medullary thyroid carcinoma (MTC), in humans as human relevance of semaglutide-induced rodent thyroid C-cell tumors has not been determined.
- Wegovy™ is contraindicated in patients with a personal or family history of MTC or in patients with Multiple Endocrine Neoplasia syndrome type 2 (MEN 2). Counsel patients regarding the potential risk for MTC with the use of Wegovy™ and inform them of symptoms of thyroid tumors (e.g. a mass in the neck, dysphagia, dyspnea, persistent hoarseness). Routine monitoring of serum calcitonin or using thyroid ultrasound is of uncertain value for early detection of MTC in patients treated with Wegovy™.

Contraindications

- Wegovy™ is contraindicated in patients with a personal or family history of MTC or in patients with MEN 2, and in patients with a prior serious hypersensitivity reaction to semaglutide or to any of the excipients in Wegovy™. Serious hypersensitivity reactions, including anaphylaxis and angioedema have been reported with semaglutide.

Warnings and Precautions

- **Risk of Thyroid C-Cell Tumors:** Patients should be further evaluated if serum calcitonin is measured and found to be elevated or thyroid nodules are noted on physical examination or neck imaging.
- **Acute Pancreatitis:** Acute pancreatitis, including fatal and non-fatal hemorrhagic or necrotizing pancreatitis, has been observed in patients treated with GLP-1 receptor agonists, including semaglutide. Acute pancreatitis was observed in patients treated with Wegovy™ in clinical trials. Observe patients carefully for signs and symptoms of acute pancreatitis (including persistent severe abdominal pain, sometimes radiating to the back, and which may or may not be accompanied by vomiting). If acute pancreatitis is suspected, discontinue Wegovy™ promptly, and if acute pancreatitis is confirmed, do not restart.
- **Acute Gallbladder Disease:** In clinical trials, cholelithiasis was reported by 1.6% of Wegovy™ patients and 0.7% of placebo patients. Cholecystitis was reported by 0.6% of Wegovy™ patients and 0.2% of placebo patients. If cholelithiasis is suspected, gallbladder studies and appropriate clinical follow-up are indicated.
- **Hypoglycemia:** Wegovy™ lowers blood glucose and can cause hypoglycemia. In a trial of patients with type 2 diabetes, hypoglycemia was reported in 6.2% of Wegovy™ patients versus 2.5% of placebo patients. Patients with type 2 diabetes taking Wegovy™ with an insulin secretagogue (e.g. sulfonylurea) or insulin may have an increased

risk of hypoglycemia, including severe hypoglycemia. Inform patients of the risk of hypoglycemia and educate them on the signs and symptoms. Monitor blood glucose in patients with type 2 diabetes.

- **Acute Kidney Injury:** There have been postmarketing reports of acute kidney injury and worsening of chronic renal failure, which in some cases required hemodialysis, in patients treated with semaglutide. Patients with renal impairment may be at a greater risk of acute kidney injury, but some events have been reported in patients without known underlying renal disease. A majority of the events occurred in patients who experienced nausea, vomiting, or diarrhea, leading to volume depletion. Monitor renal function when initiating or escalating doses of Wegovy™ in patients reporting severe adverse gastrointestinal reactions and in patients with renal impairment reporting any adverse reactions that could lead to volume depletion.
- **Hypersensitivity:** Serious hypersensitivity reactions (e.g., anaphylaxis, angioedema) have been reported with semaglutide. If hypersensitivity reactions occur, discontinue use of Wegovy™, treat promptly per standard of care, and monitor until signs and symptoms resolve. Use caution in a patient with a history of anaphylaxis or angioedema with another GLP-1 receptor agonist.
- **Diabetic Retinopathy Complications in Patients with Type 2 Diabetes:** In a trial of patients with type 2 diabetes, diabetic retinopathy was reported by 4.0% of Wegovy™ patients and 2.7% of placebo patients. Rapid improvement in glucose control has been associated with a temporary worsening of diabetic retinopathy. Patients with a history of diabetic retinopathy should be monitored for progression of diabetic retinopathy.
- **Heart Rate Increase:** Mean increases in resting heart rate of 1 to 4 beats per minute (bpm) were observed in Wegovy™ patients compared to placebo in clinical trials. More Wegovy™ patients compared with placebo had maximum changes from baseline of 10 to 19 bpm (41% versus 34%) and 20 bpm or more (26% versus 16%). Monitor heart rate at regular intervals and instruct patients to report palpitations or feelings of a racing heartbeat while at rest. If patients experience a sustained increase in resting heart rate, discontinue Wegovy™.
- **Suicidal Behavior and Ideation:** Suicidal behavior and ideation have been reported in clinical trials with other weight management products. Monitor patients for depression, suicidal thoughts or behavior, and/or any unusual changes in mood or behavior. Discontinue Wegovy™ in patients who experience suicidal thoughts or behaviors and avoid in patients with a history of suicidal attempts or active suicidal ideation.

Adverse Reactions

- The most common adverse reactions reported in $\geq 5\%$ of patients treated with Wegovy™ are nausea, diarrhea, vomiting, constipation, abdominal pain, headache, fatigue, dyspepsia, dizziness, abdominal distention, eructation, hypoglycemia in patients with type 2 diabetes, flatulence, gastroenteritis, and gastroesophageal reflux disease.

Drug Interactions

- The addition of Wegovy™ in patients treated with insulin has not been evaluated. When initiating Wegovy™, consider reducing the dose of concomitantly administered insulin secretagogues (such as sulfonylureas) or insulin to reduce the risk of hypoglycemia.
- Wegovy™ causes a delay of gastric emptying and has the potential to impact the absorption of concomitantly administered oral medications. Monitor the effects of oral medications concomitantly administered with Wegovy™.

Use in Specific Populations

- **Pregnancy:** May cause fetal harm. When pregnancy is recognized, discontinue Wegovy™. Discontinue Wegovy™ in patients at least 2 months before a planned pregnancy.

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Mechanisms of *Artemisia scoparia*'s Anti-Inflammatory Activity in Cultured Adipocytes, Macrophages, and Pancreatic β -Cells

Anik Boudreau ¹, Susan J. Burke ¹, J. Jason Collier ¹, Allison J. Richard ¹, David M. Ribnicky³, and Jacqueline M. Stephens ^{1,2}

Objective: An ethanolic extract of *Artemisia scoparia* (SCO) improves adipose tissue function and reduces negative metabolic consequences of high-fat feeding. *A. scoparia* has a long history of medicinal use across Asia and has anti-inflammatory effects in various cell types and disease models. The objective of the current study was to investigate SCO's effects on inflammation in cells relevant to metabolic health.

Methods: Inflammatory responses were assayed in cultured adipocytes, macrophages, and insulinoma cells by quantitative polymerase chain reaction, immunoblotting, and NF- κ B reporter assays.

Results: In tumor necrosis factor α -treated adipocytes, SCO mitigated ERK and NF- κ B signaling as well as transcriptional responses but had no effect on fatty acid-binding protein 4 secretion. SCO also reduced levels of deleted in breast cancer 1 protein in adipocytes and inhibited inflammatory gene expression in stimulated macrophages. Finally, in pancreatic β -cells, SCO decreased NF- κ B-responsive promoter activity induced by IL-1 β treatment.

Conclusions: SCO's ability to promote adipocyte development and function is thought to mediate its insulin-sensitizing actions *in vivo*. Our findings that SCO inhibits inflammatory responses through at least two distinct signaling pathways (ERK and NF- κ B) in three cell types known to contribute to metabolic disease reveal that SCO may act more broadly than previously thought to improve metabolic health.

Obesity (2020) **28**, 1726–1735.

Introduction

The obesity epidemic and its associated metabolic disorders are among the major health challenges of our time (1). Adipose tissue plays a crucial role in these metabolic disease states, and disruptions in adipocyte development and function negatively impact whole-body insulin sensitivity and systemic metabolic health (2). Adipocyte differentiation and adipose tissue expansion are often impaired in obese states, leading to insulin resistance and metabolic dysregulation (3,4). In addition, obesity and insulin resistance are associated with enhanced basal lipolysis rates, which exacerbate metabolic disease (reviewed in Morigny et al.) (5). Inflammatory processes in adipose tissue are also a prominent feature of obesity and the metabolic syndrome, and there is extensive cross talk between inflammation, adipogenesis,

Study Importance

What is already known?

- Adipose tissue inflammation plays a critical role in whole-body metabolic function in the context of obesity.
- An ethanolic extract of *Artemisia scoparia* (SCO) improves measures of adipocyte and adipose tissue function as well as whole-body insulin sensitivity in a mouse model of diet-induced obesity.

What does this study add?

- SCO inhibits inflammatory gene expression in cultured adipocytes and macrophages.
- SCO inhibits ERK signaling in adipocytes as well as NF- κ B signaling in adipocytes and pancreatic β -cells.

How might these results change the direction of research?

- SCO's effects on adipocyte function and development have been well studied. Our current results indicate that SCO may be acting on various cell types, through several mechanisms, to mitigate obesity-related metabolic dysfunction; this opens up new directions for the investigation of SCO's properties.
- Our new data support the further study of SCO as a dietary supplement to promote metabolic resilience.

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endocrine function, and lipid metabolism in adipose tissue (5,6). Because of its importance in obesity and diabetes, adipose tissue is considered a valuable target for therapeutic intervention (2).

Botanicals have an extensive history of medicinal use across most, if not all, cultures the world over, and plants have long been an important source of pharmaceutical compounds. Notably, metformin, the first-line drug in treating type 2 diabetes mellitus, was derived from galegine, a bioactive first isolated from *Galega officinalis*, also known as goat's rue or French lilac (7). Screening efforts in our laboratory identified an extract of *Artemisia scoparia* (SCO) as a potent enhancer of adipocyte differentiation *in vitro*. Subsequent studies in a diet-induced obesity (DIO) mouse model have shown that SCO also has metabolically beneficial effects on adipose tissue *in vivo* (8,9). In addition, SCO supplementation improves whole-body insulin sensitivity and reduces circulating levels of fatty acids and triglycerides (8-10). More recently, we demonstrated that SCO can act on adipocytes *in vitro* to reduce lipolysis induced by the inflammatory cytokine tumor necrosis factor alpha (TNF α) (11).

A. scoparia has a well-documented history of medicinal use (12,13), and it has been shown to have a wide range of effects in disease models related to Alzheimer disease, renal oxidative stress, hepatotoxicity, hypertension, and others (14-17). More specifically, anti-inflammatory effects of *A. scoparia* have been described in a wide range of cell types and contexts (18-20). In adipose tissue, TNF α secreted from resident macrophages is a principal mediator of obesity-associated inflammation (6,21,22). Given the important role of adipose tissue inflammation in obesity-related metabolic dysfunction, we examined the ability of SCO to regulate TNF α action and inflammatory gene expression in adipocytes. We observed that SCO reduced effects of TNF α on inflammatory cytokine expression and nuclear factor- κ B (NF- κ B) activation. SCO also reduced nuclear levels of deleted in breast cancer 1 (DBC1, also known as cell cycle and apoptosis regulator 2 [CCAR2]), a protein associated with impaired metabolic function (23-26). Although SCO reduced lipolysis induced by TNF α , it had no effect on TNF α -induced secretion of fatty acid-binding protein 4 (FABP4), which has been shown to be enhanced by lipolytic stimuli and reduced by interventions that inhibit lipolysis (27,28). Finally, we observed anti-inflammatory effects of SCO in two other cell types critical in metabolic disease states: macrophages and pancreatic β -cells. Taken together, data presented herein, as well as previously published studies, support further investigation of SCO as a potential therapeutic in the treatment of metabolic disease.

Methods

Botanical extract source and preparation

A. scoparia plants were grown and harvested, and ethanolic extracts were prepared as previously reported (8,11). SCO extracts were dissolved in dimethyl sulfoxide (DMSO) at 1000X final concentration (10 mg/mL and 50 mg/mL, respectively, for 10- or 50- μ g/mL treatments).

Adipocyte cell culture and treatments

3T3-L1 preadipocytes were grown and differentiated as previously described (8). Briefly, cells were grown in high-glucose DMEM supplemented with 10% bovine calf serum. Cells were induced to differentiate 2 days after reaching confluence in DMEM plus 10% fetal bovine serum (FBS) containing 0.5mM 3-isobutyl-1-methylxanthine (IBMX), 1 μ M

dexamethasone, and 1.7 μ M insulin. Cells were fed 48 to 72 hours later with DMEM plus 10% FBS and 0.425 μ M insulin and then every 48 to 72 hours with DMEM plus 10% FBS. DMEM, IBMX, dexamethasone, and insulin were obtained from Sigma-Aldrich (St. Louis, Missouri); calf serum and FBS were purchased from Hyclone (GE Life Sciences, Logan, Utah). Treatments with SCO were initiated between 6 and 13 days after induction of differentiation. Murine TNF α was purchased from Life Technologies (Carlsbad, California), dissolved at 0.5 μ M in phosphate-buffered saline (PBS) containing 0.1% bovine serum albumin (BSA), and added to cell culture media at the final concentrations indicated in figure legends.

Macrophage cell culture and treatments

RAW 264.7 macrophages were cultured in high-glucose DMEM supplemented with 10% FBS. Cells were pretreated for 2 hours with SCO, and then lipopolysaccharide (LPS) from *Escherichia coli* 0111:B4 strain (InvivoGen, San Diego, California) was added for an additional 5.5-hour incubation before harvest.

Insulinoma cell culture and treatments

The 832/13 rat insulinoma cells were cultured as previously described (29) and transfected with an adenoviral luciferase reporter construct under the control of five copies of a consensus NF- κ B element (vector obtained from Vector Biolabs, catalog number 1740; Malvern, Pennsylvania). A total of 12 hours after transduction, cells were treated overnight with 5 or 10 μ g/mL of SCO or *Artemisia santolinaefolia* and then for 4 hours with 1 ng/mL of interleukin (IL)-1 β . Luciferase reporter activity was assessed by luminometry and normalized to total protein content.

RNA isolation and gene expression analysis

Treated cells were harvested in cell lysis RLT Buffer (Qiagen, Hilden, Germany). Lysates were stored at -80°C prior to RNA extraction using the RNeasy Mini Kit (Qiagen). The High-Capacity Reverse Transcription Kit (Applied Biosystems, Foster City, California) was used to reverse transcribe the RNA samples, and gene expression was assayed by quantitative polymerase chain reaction (PCR) with Takara SYBR premix (Takara Bio USA, Mountain View, California) on the Applied Biosystems 7900HT system (cycling conditions: 2 minutes at 50°C; 10 minutes at 95°C; 40 cycles of 15 seconds at 95°C and 1 minute at 60°C; dissociation stage: 15 seconds at 95°C, 15 seconds at 60°C, and 15 seconds at 95°C with end step ramp rate of 2%). Data were analyzed using SDS 2.3 or 2.4 software (Applied Biosystems, Foster City, California). Target gene data were normalized to the reference gene non-POU-domain-containing, octamer-binding (*Nono*). Primers were obtained from Integrated DNA Technologies (Skokie, Illinois), and sequences were as follows: *Nono* forward: 5'-CATCATCAGCATCACCACCA-3', reverse: 5'-TCTTCAGGTCAATAGTCAAGCC-3'; C-C motif chemokine ligand 2 (*Ccl2*, also known as monocyte chemoattractant protein 1 [*Mcp1*]) forward: 5'-GCAGAGAGCCAGACGGGAGGA-3', reverse: 5'-TGGGG CGTAACTGCATCTGG-3'; *I6* forward: 5'-TCCTCTCTGCAAGA GACTTCCATCC-3', reverse: 5'-AAGCCTCCGACTTGTGAAGTG GT-3'; lipocalin 2 (*Lcn2*) forward: 5'-TGCAAGTGGCCACCACG GAC-3', reverse: 5'-GCATTGGTCCGGTGGGACAGAGA-3'; nitric oxide synthase 2 inducible (*Nos2* or *iNos*) forward: 5'-CCCTCC TGATCTTGTGTTGGA-3', reverse: 5'-TCAACCCGAGCTCCTG GAA-3'; *Tnfa* forward: 5'-AGACCCTCACACTCAGATCA-3', reverse: 5'-TCTTTGAGATCCATGCCGTTG-3'.

Protein samples and immunoblotting

For whole-cell extract preparation, adipocyte monolayers were harvested in radioimmunoprecipitation assay buffer (30) containing the following protease and phosphatase inhibitors: 1mM phenylmethylsulfonyl fluoride (PMSF), 1μM pepstatin, 50 trypsin inhibitory milliunits of aprotinin, 10μM leupeptin, 1mM 10-phenanthroline, 0.2mM sodium orthovanadate, and 100μM sodium fluoride. Lysates were stored at -80°C and then thawed, passed through a 20 gauge needle three times, and clarified by centrifugation at 13,000g. Supernatants were recovered, and protein concentrations were determined by bicinchoninic acid assay (Sigma-Aldrich). Equal amounts of protein from each sample were loaded onto polyacrylamide gels, subjected to electrophoresis, and transferred to nitrocellulose. Standard immunoblotting techniques were applied to probe for target proteins. Primary antibodies were purchased from Cell Signaling Technologies (Danvers, Massachusetts) for extracellular signal-regulated kinase (ERK) 1/2, CCAR2 (DBC1), β-actin, and NF-κB p65; from R&D Systems (Bio-Techne, Minneapolis, Minnesota) for LCN2; from Promega (Madison, Wisconsin) for phosphorylated ERK 1/2 (active mitogen-activated protein kinase, or MAPK); and from Abcam (Cambridge, Massachusetts) for FABP4. Detection was performed with horseradish peroxidase-conjugated antibodies from Jackson ImmunoResearch (West Grove, Pennsylvania). Autoradiography films were scanned and densitometry analysis performed using ImageStudio software from Li-Cor Biosciences (Lincoln, Nebraska).

Subcellular fractionation

After treatment, adipocytes were harvested in nuclear homogenization buffer (NHB) (20mM Tris pH 7.4, 10mM NaCl, and 3mM MgCl₂). IGEPAL CA-630 (Sigma-Aldrich) was added to the cell suspension at a final concentration of 0.15% prior to Dounce homogenization on ice. The nuclear fraction was pelleted by centrifugation at 517g, washed in NHB, and resuspended in immunoprecipitation buffer. NHB and immunoprecipitation buffer were supplemented with protease and phosphatase inhibitors. Protein concentrations were determined by bicinchoninic acid assay.

FABP4 secretion and lipolysis assays

Cells pretreated for 3 days with SCO or DMSO vehicle were treated overnight with or without 0.75nM TNFα. The following morning, culture medium was replaced with lipolysis incubation medium (low-glucose DMEM + 2% BSA). Cells treated with TNFα overnight were treated with the same concentration of TNFα for the lipolysis/FABP4 secretion analysis. The remaining cells were treated with either vehicle (controls) or 2nM or 10μM isoproterenol. Conditioned media samples were collected after 4 hours and analyzed for FABP4 content by immunoblotting. Lipolysis was also assessed by measuring glycerol concentrations in the samples, using free glycerol reagent from Sigma-Aldrich.

Results

SCO inhibits TNFα-induced inflammatory gene expression in 3T3-L1 adipocytes

Our previous studies have demonstrated that SCO could reduce protein levels of the inflammatory cytokine CCL2 (also known as MCP-1) in the adipose tissue of high-fat diet-fed mice and inflammatory gene expression in cultured adipocytes (9). Hence, we have sought to characterize the cell-autonomous anti-inflammatory effects of SCO in 3T3-L1 adipocytes treated with TNFα, a predominant mediator of adipose

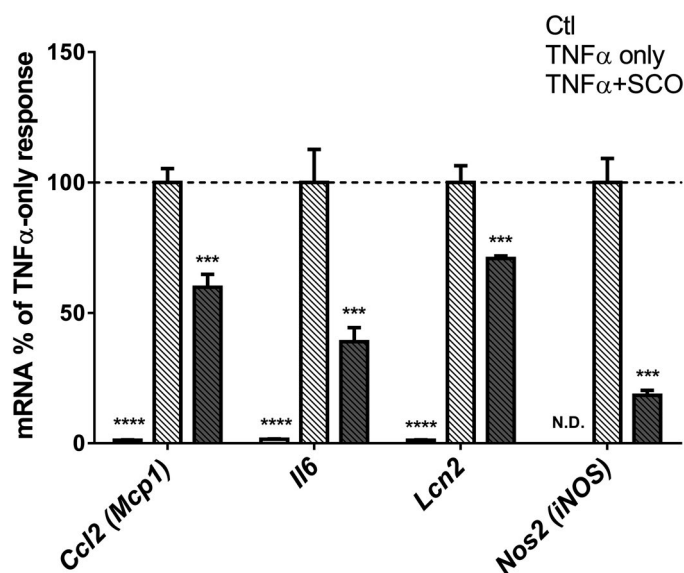


Figure 1 SCO inhibits TNFα-induced expression of inflammatory genes in 3T3-L1 adipocytes. Mature 3T3-L1 adipocytes were pretreated with 50 μg/mL of SCO for 3 days and then with 0.75nM TNFα for 18 hours. Cells were harvested for RNA isolation, and gene expression was assayed by quantitative PCR. Target gene data were normalized to the reference gene *Nono* and expressed as percent of TNFα-induced values. Data are shown as means ± SE; *n* = 4 biological replicates per condition. N.D.: *Nos2* was below detection level for the quantitative PCR assay. Data for each gene were analyzed by one-way ANOVA (for *Ccl2*, *Il6*, and *Lcn2*) or *t* test (for *Nos2*). Significance was expressed as ****P* < 0.001, *****P* < 0.0001 vs. TNFα-only controls. Results were replicated in two additional experiments on independent batches of cells. Ctl, vehicle-only control.

tissue inflammation that increases the expression of several inflammatory genes in adipocytes (21). As shown in Figure 1, pretreatment of 3T3-L1 adipocytes with 50 μg/mL of SCO significantly diminished TNFα-induced expression of *Ccl2* and *Il6*, consistent with previously published studies (8), as well as *Lcn2* and *Nos2* or (*iNos*). We also examined the time course for induction of these genes and for the effects of SCO. As shown in Figure 2, the temporal patterns of induction by TNFα and inhibition by SCO were different for all four genes assayed. While *Ccl2* and *Lcn2* both increased gradually over the 8-hour TNFα treatment, the effects of SCO were distinct. In the case of *Ccl2*, SCO significantly inhibited TNFα-induced gene expression at all time points. Yet, for *Lcn2*, SCO increased expression in basal conditions at all time points and at the early time points of TNFα treatment (1 and 2 hours). After 4 hours of TNFα treatment, SCO-treated cells had equivalent *Lcn2* expression to the controls, and after 8 hours, expression was significantly lower in SCO-treated cells. Induction of *Il6* gene expression by TNFα was observed at all time points, but the response was biphasic. Specifically, expression strongly increased at 1 hour, subsided at 2 and 4 hours, and then substantially increased again at 8 hours. This same pattern of TNFα induction was observed in SCO-treated cells but with lower *Il6* expression levels than without SCO at all time points. SCO attenuation of the TNFα effect on *Il6* was significant at 1, 4, and 8 hours but not at 2 hours. Finally, *Nos2* expression and nitric oxide production induced by TNFα have been implicated in the regulation of adipocyte lipolysis (31). TNFα induced *Nos2* expression starting at the 2-hour time point, and SCO significantly but modestly inhibited TNFα's effects, consistent with its previously described antilipolytic actions (11).

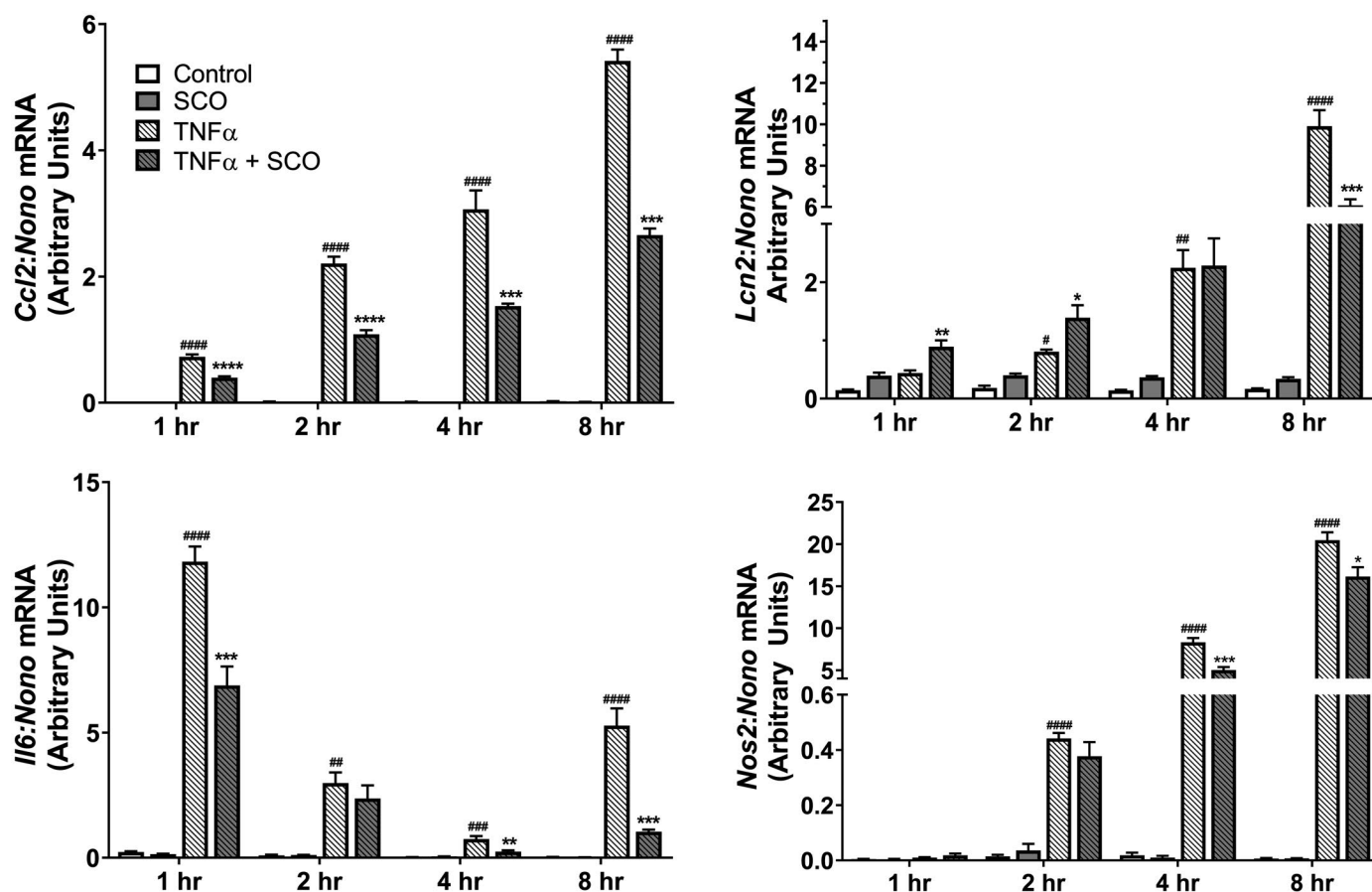


Figure 2 Time course of TNF α - and SCO-induced changes in inflammatory gene expression. Mature 3T3-L1 adipocytes were pretreated with 50 μ g/mL of SCO or DMSO vehicle for 3 days prior to harvest. Media was replaced with low-glucose DMEM supplemented with 2% BSA, and cells were treated with 0.75 nM TNF α or 0.1% BSA vehicle for the times indicated and harvested for RNA isolation. Expression of the inflammatory genes was assessed by quantitative PCR and normalized to the reference gene *Nono*. One-way ANOVA was performed for each time point. ## P <0.01, ### P <0.001, and #### P <0.0001, effect of TNF α vs. control; * P <0.05, ** P <0.01, *** P <0.001, and **** P <0.0001, effect of SCO vs. respective controls (control or TNF α only). Results were replicated on an independent batch of cells.

SCO inhibits TNF α -induced LCN2 secretion in 3T3-L1 adipocytes

LCN2 is a proinflammatory mediator secreted by adipocytes (reviewed in Li et al.) (32). We have previously shown that LCN2 expression and secretion are induced by TNF α in 3T3-L1 adipocytes (33). As shown in Figure 3, the TNF α -induced expression and secretion of LCN2 from adipocytes were substantially reduced in the presence of SCO. These observations are consistent with the ability of SCO to impair TNF α induction of *Lcn2* gene expression (Figures 1-2).

SCO reduces total and phosphorylated ERK levels

In adipocytes, TNF α signaling mediates transcriptional regulation of numerous target genes involved in adipocyte function (inflammation, insulin signaling, lipolysis, endocrine function, stress responses) through the activation of several signaling pathways (reviewed in Cawthorn et al.) (21), including the MAPK ERK. To further characterize the effects of SCO in the context of TNF α action in adipocytes, we examined ERK activation in the presence or absence of SCO. As

shown in Figure 4, TNF α regulates both the expression of ERK and its activation, as judged by phosphorylation. TNF α induces ERK phosphorylation, and this activation is reduced in the presence of SCO. We also observed that total ERK1/2 levels were modulated by both TNF α (increased) and SCO (decreased). Comparison of the ratios of phosphorylated ERK 1/2 to total ERK 1/2 revealed that the effect of SCO on relative ERK activation was not significant.

SCO inhibits TNF α -induced nuclear translocation of NF- κ B and reduces nuclear levels of DBC1 in 3T3-L1 adipocytes

Another major signaling pathway engaged by TNF α in adipocytes is the inhibitor of nuclear factor- κ B kinase (IKK)/NF- κ B pathway. TNF α causes phosphorylation of the IKK complex as well as phosphorylation and degradation of its target, inhibitor of NF- κ B, resulting in the translocation of NF- κ B to the nucleus. In order to determine whether SCO could modulate the TNF α activation of NF- κ B in fat cells, we treated 3T3-L1 adipocytes with TNF α , with or without a 3-day SCO pretreatment. The cytosolic and nuclear compartments were analyzed

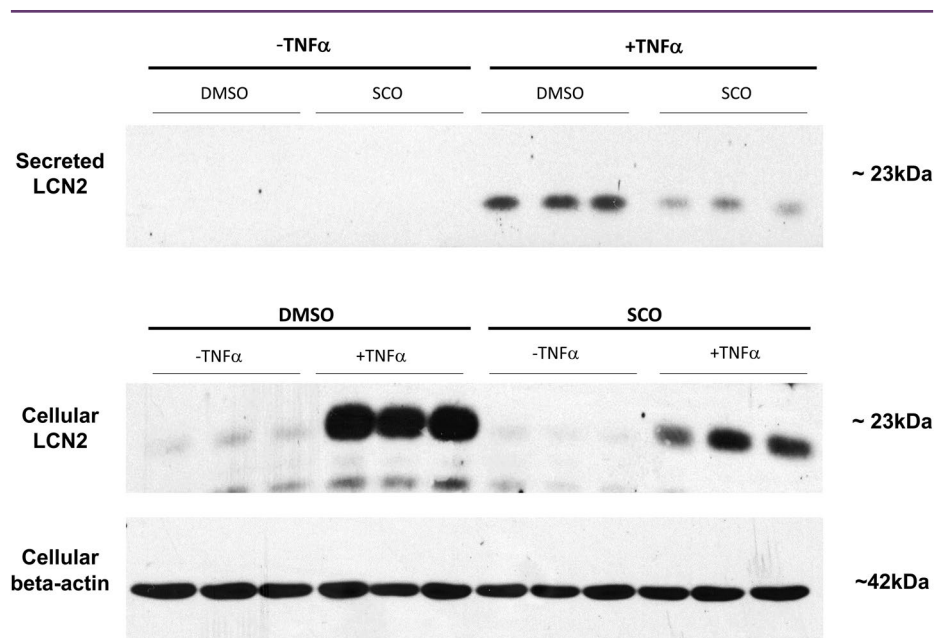


Figure 3 SCO inhibits TNF α -induced cellular levels and secretion of LCN2 in adipocytes. Mature 3T3-L1 adipocytes were pretreated with 50 μ g/mL of SCO or DMSO vehicle for 3 days prior to harvest. On the last day of SCO treatment, cells were treated with 0.75nM TNF α or 0.1% BSA vehicle for 18 hours. Medium on all cells was then replaced with low-glucose phenol-red-free DMEM containing either vehicle or 0.75nM TNF α . After 4 hours, samples of conditioned media were collected, and cells were harvested in radioimmunoprecipitation assay buffer for immunoblotting. A total of 125 μ g (media) or 75 μ g (whole-cell extracts) of total protein was loaded in each well.

by immunoblotting. As shown in Figure 5, TNF α treatment produced a very robust increase in nuclear levels of the NF- κ B p65 subunit, and SCO pretreatment greatly inhibited this response, indicating the ability of SCO to interfere with a major inflammatory signaling event.

The nuclear protein DBC1, also known as CCAR2, has been implicated in several processes related to inflammation, adipocyte biology, and insulin resistance (23-25). Recent work in our laboratory has described a role for DBC1 in regulating TNF α -induced lipolysis in 3T3-L1 adipocytes (26). We examined DBC1 protein levels in cytosolic and nuclear fractions in TNF α -treated cells in the presence and absence of SCO (Figure 5) and found that SCO-treated adipocytes had less nuclear DBC1 in both basal and TNF α -stimulated conditions. As previously reported, DBC1 is not detected in the cytosol of cultured adipocytes (26).

SCO does not reduce TNF α - or isoproterenol-induced FABP4 secretion

In addition to inducing expression of inflammatory mediators in adipocytes, TNF α also stimulates lipolysis, thereby increasing circulating fatty acid levels and promoting further metabolic dysregulation in obesity and insulin-resistant states (5). We have previously shown that SCO inhibits TNF α -induced but not adrenergic-stimulated lipolysis in adipocytes (11). FABP4 is secreted from adipocytes and adipose tissue in response to lipolytic conditions, including exposure to forskolin, cyclic adenosine monophosphate (cAMP), or isoproterenol (27,28). To our knowledge, the effects of TNF α on FABP4 secretion have not been reported. Therefore, we induced lipolysis in adipocytes using TNF α or isoproterenol, with or without SCO pretreatment, and measured FABP4

levels in the conditioned medium to assess whether TNF α could induce secretion of FABP4 in 3T3-L1 adipocytes under prolipolytic conditions. Consistent with previous studies, both doses of isoproterenol tested stimulated FABP4 secretion (Figure 6A). We also made the novel observation that TNF α promoted secretion of FABP4 in the presence and absence of SCO (Figure 6A). Additionally, we examined lipolysis, as measured by glycerol release, in these adipocytes. As shown in Figure 6B, SCO did not attenuate isoproterenol-induced lipolysis but did attenuate TNF α -induced lipolysis. SCO had no effect on FABP4 secretion in any of the treatments (Figure 6A). Of note, SCO significantly reduced lipolysis, but not FABP4 secretion, in TNF α -treated adipocytes.

SCO reduces LPS-induced expression of *Il1b* and *Nos2* (*iNos*) but not *Tnfa* in macrophages

Adipose tissue macrophages promote inflammation and contribute to adipocyte dysfunction in obesity and insulin-resistant states (6,22). Hence, we examined the ability of SCO to modulate inflammatory gene expression in RAW 264.7 cells treated with LPS. As shown in Figure 7, LPS elicited a robust induction of *Tnfa*, *Il1b*, and *Nos2*. SCO pretreatment inhibited the LPS effect on *Il1b* and *Nos2* in a dose-dependent manner but had no effect on the induction of *Tnfa* gene expression by LPS, revealing a gene-specific anti-inflammatory effect of SCO in these cultured macrophages.

SCO reduces IL-1 β -induced NF- κ B activation in cultured pancreatic beta cells

NF- κ B signaling is crucial in mediating the inflammatory transcriptional responses that contribute to pancreatic β -cell dysfunction in

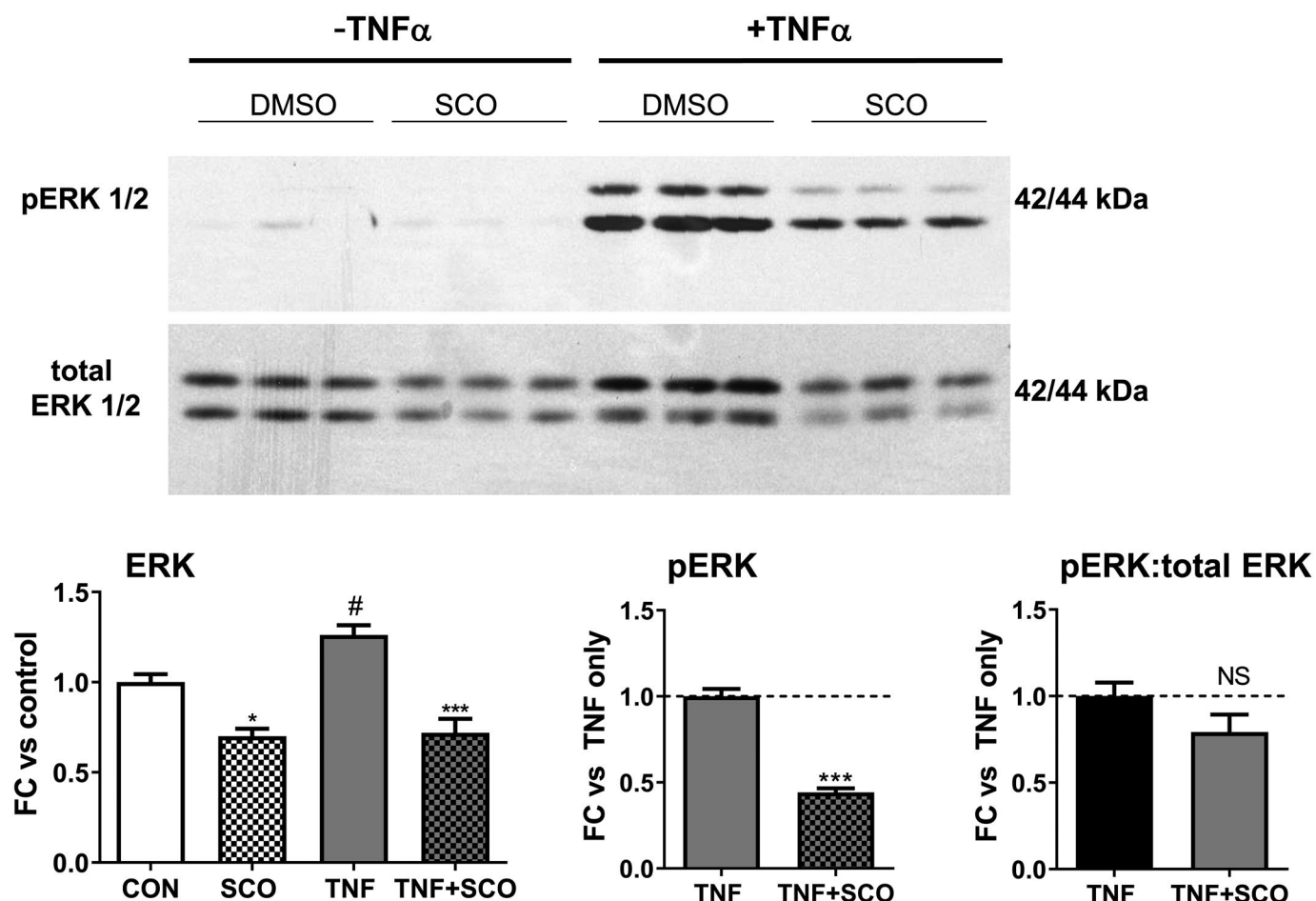


Figure 4 SCO reduces total and phosphorylated ERK levels in 3T3-L1 adipocytes. Mature 3T3-L1 adipocytes were pretreated with 50 μ g/mL of SCO or DMSO vehicle for 3 days, with 18-hour (overnight) serum deprivation (0.3% BSA) and 0.5nM TNF α treatment on the last day. Cells were harvested in radioimmunoprecipitation assay buffer and analyzed by Western blot. Blot images and quantitation data are shown. Data expressed as foldchange (FC) vs. control (CON). * P <0.05 and *** P <0.001, effect of SCO vs. respective controls (CON or TNF α only); # P <0.05, effect of TNF α vs. CON. NS, not significant. Results were replicated in two additional experiments on independent batches of cells.

diabetes (34-38). To determine whether SCO could regulate NF- κ B activation in β -cells, we transduced 823/13 rat insulinoma cells with a NF- κ B luciferase reporter and then treated cells with SCO at 5 or 10 μ g/mL or vehicle overnight before stimulating cells with IL-1 β for 4 hours. IL-1 β treatment induced NF- κ B promoter activation 28.6-fold over untreated controls. As shown in Figure 8, we observed a dose-dependent reduction in NF- κ B promoter activity in the presence of SCO. The higher dose of SCO resulted in a statistically significant decrease in IL-1 β -induced NF- κ B promoter activity. This effect was not observed with an extract from *A. santolinaefolia*, a different *Artemisia* species also known to have adipogenic effects *in vitro* as well as some metabolically favorable effects in a mouse DIO model (9).

Discussion

The impacts of obesity, metabolic syndrome, and T2DM clearly justify the search for novel therapeutic approaches, and adipose tissue is intensely studied as a target for such interventions. Obesity is considered a proinflammatory state, and the importance of adipose tissue

inflammation in the progression of insulin resistance is well documented. In obesity, infiltration and activation of proinflammatory immune cells (particularly macrophages) in adipose tissue contribute to impaired adipocyte differentiation and function as well as to systemic insulin resistance, mediated in large part by the paracrine actions of macrophage-derived TNF α on adipocytes (6,21,22). The present study demonstrates that SCO can impede inflammatory processes through at least two different signaling pathways (MAPK and NF- κ B) and in three cell types relevant to metabolic health (adipocytes, macrophages, and pancreatic β -cells).

In adipocytes, we discovered gene-specific temporal patterns of induction by TNF α and inhibition by SCO. For example, three of the four genes we examined showed a steady increase over an 8-hour TNF α treatment, while the fourth gene, *Il6*, showed a biphasic response (Figure 2). Also, though SCO reduced expression of *Il6*, *Mcp1*, and *iNos* in all TNF α -treated conditions, SCO pretreatment increased *Lcn2* expression in basal conditions and at the early time points of TNF α treatment but was inhibitory with longer TNF α treatments. Given the low levels of *Lcn2* expression in basal conditions and at early time

points of TNF α induction, SCO's effects on these inflammatory genes under basal conditions are not likely to have physiological relevance. Likewise, SCO's inhibition of *Nos2* expression is modest after 2, 4, or 8 hours of TNF α treatment compared with the robust induction with TNF α and may not be of any biological significance. Taken together,

these observations underscore the complexity of inflammatory signaling and suggest that SCO may be acting through distinct mechanisms on different genes.

Activation of the MAPK ERK by phosphorylation mediates some of the effects of TNF α in adipocytes (21). Although phosphorylated to total ERK1/2 ratios were not significantly altered by SCO pretreatment, TNF α -treated cells had lower absolute levels of active phosphorylated ERK 1/2 when pretreated with SCO (Figure 4), indicating suppression of MAPK signaling. This effect on ERK activation could contribute to the SCO-mediated reductions in inflammatory gene expression we observed, particularly that of *Lcn2*, whose expression has been shown to be dependent on ERK 1/2 activation (39). The ability of SCO to decrease total levels of ERK 1/2 implies that SCO pretreatment may alter its transcription, translation, or stability. Avenues for further investigation of this observation could include studies of protein stability, unfolded protein response, and endoplasmic reticulum stress, among others. At this time, we do not know whether the effect of SCO on ERK expression is required for its anti-inflammatory actions.

The NF- κ B pathway is also well characterized and known to mediate TNF α 's effects on inflammatory cytokine gene transcription through nuclear translocation of the p65 subunit of NF- κ B, and NF- κ B signaling events are involved in inflammation-related metabolic disease (reviewed in Baker et al.) (40). A study in LPS-stimulated macrophages has shown that the total flavonoid fraction of *A. scoparia* could inhibit inflammatory signaling through MAPK and NF- κ B (19). Our studies in adipocytes reveal that SCO caused substantial inhibition of both pathways in adipocytes and provide further evidence that SCO impedes inflammatory signaling pathways in fat cells.

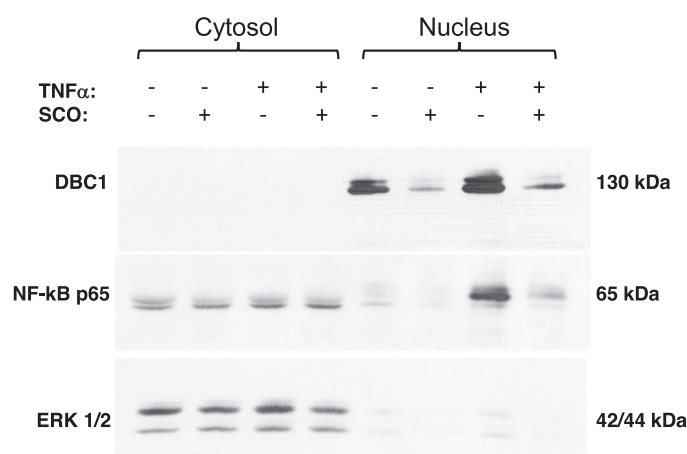


Figure 5 SCO reduces nuclear DBC1 and TNF α -induced p65 nuclear translocation in 3T3-L1 adipocytes. Mature 3T3-L1 adipocytes were pretreated for 3 days with 50 μ g/mL of SCO or DMSO vehicle and then with 0.5nM TNF α for 20 minutes. Cells were harvested, and the cytosolic and nuclear compartments were isolated. Immunoblotting was performed to detect DBC1 and the p65 subunit of NF- κ B. ERK 1/2 was included as a loading control for the cytoplasmic fractions.

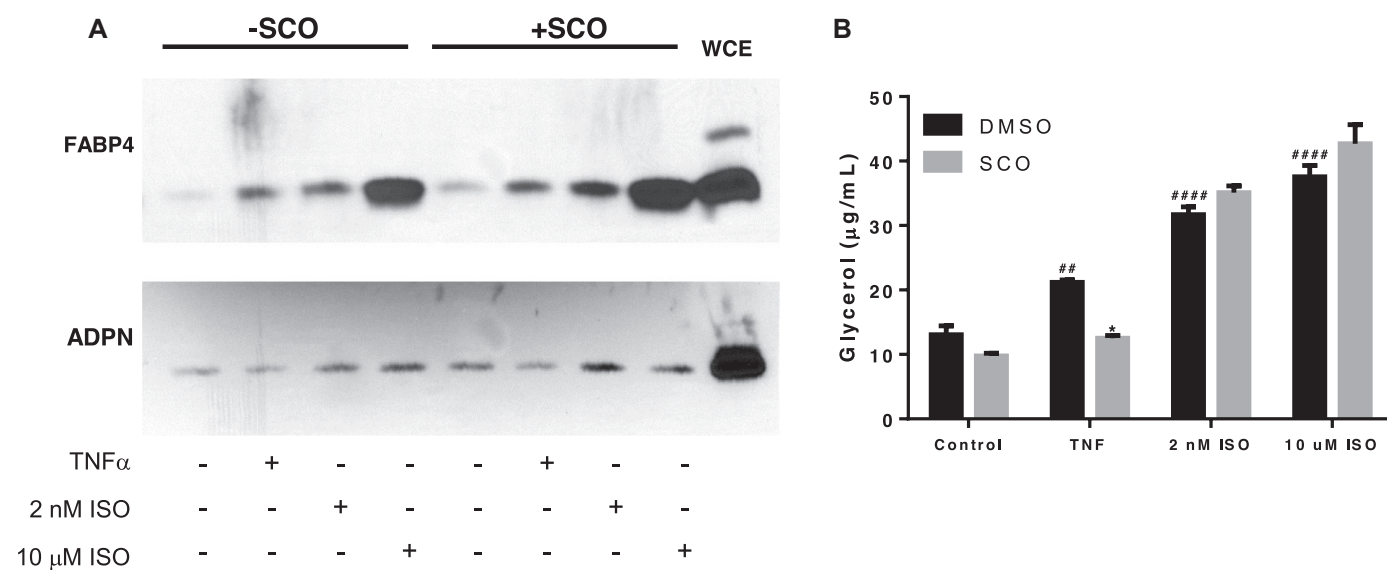


Figure 6 SCO does not reduce TNF α - or isoproterenol-induced FABP4 secretion. Mature 3T3-L1 adipocytes were pretreated with 50 μ g/mL of SCO or DMSO vehicle for 3 days prior to harvest. On the last day of SCO treatment, cells were treated with 0.75nM or 0.1% BSA vehicle overnight (18 hours). The following day, medium on all cells was replaced with low-glucose phenol-red-free DMEM containing either vehicle, 0.75nM TNF α , or 2nM or 10 μ M isoproterenol (ISO). Samples of conditioned media were collected after 4 hours. (A) Total protein (75 μ g) was loaded in each well and analyzed with standard immunoblotting techniques. Membranes were probed for FABP4; adiponectin (ADPN) was used as a loading control. Blot images are shown. (B) Conditioned media (50 μ L) was also assayed for glycerol content. Effects of TNF α and ISO vs. control in the DMSO condition were analyzed by one-way ANOVA; ** P <0.01 and **** P <0.0001 vs. DMSO control. Effect of SCO was assessed in each condition vs. the respective DMSO condition by t tests; * P <0.05. All other interactions were nonsignificant. WCE, whole-cell extract control lysate.

DBC1 is a multi-functional protein proposed to be at the interface between aging, cancer, and metabolism (41). Loss of DBC1 in adipocytes inhibits TNF α -induced lipolysis (26), and DBC1 knockout mice

are protected from several metabolic effects of DIO (23). In addition, DBC1 knockdown in adipocytes promotes adipogenesis and reduces inflammatory cytokine expression (24,25), whereas in B cells, DBC1 has been shown to interact with proteins of the IKK complex, which directly regulates NF- κ B signaling (42). Taken together, these studies link DBC1 inhibition with favorable metabolic effects. We have shown that SCO reduces DBC1 protein levels in adipocytes (Figure 5), which could contribute to its anti-inflammatory, antilipolytic, and antidiabetic properties. However, future studies will be needed to determine if loss of DBC1 plays an important role in the ability of SCO to promote metabolic health.

Elevated plasma levels of FABP4 are associated with obesity and insulin resistance in both mice and humans, and inhibition of plasma FABP4 activity improves glucose homeostasis in DIO mice (43). Acute activation of cAMP- or cyclic guanosine monophosphate (cGMP)-dependent protein kinases (PKA and PKG, respectively) by increased intracellular cAMP or cGMP levels, respectively, stimulates lipolysis and has been shown to promote FABP4 secretion from adipocytes (28,44). The mechanisms involved in the lipolytic effects of TNF α have not been fully elucidated, but they are distinct from those induced by fasting or adrenergic stimulation (5,21). We report here that induction of lipolysis by TNF α is associated with increased FABP4 secretion (Figure 6A). Though suppression of lipolysis with insulin or through pharmacological inhibition of PKA, PKG, or hormone-sensitive lipase has been shown to reduce FABP4 secretion in PKA- or PKG-stimulated conditions (27), we observed that SCO had no effect of TNF α -induced FABP4 secretion (Figure 6B) despite its ability to inhibit lipolysis, indicating that FABP4 secretion is not necessarily coupled to lipolysis. These data also argue against the involvement of FABP4 secretion in SCO's insulin-sensitizing effects.

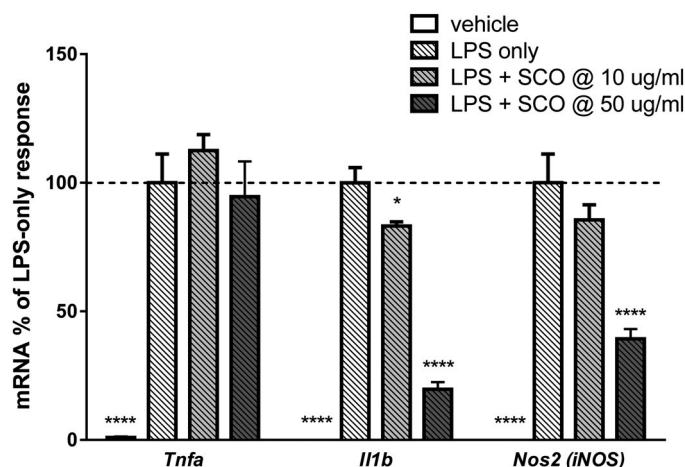


Figure 7 SCO reduces LPS-induced expression of *Il1b* and *Nos2* (*iNOS*) but not *Tnfa* in RAW 264.7 macrophages. RAW macrophages were pretreated with 10 or 50 μ g/mL of SCO for 2 hours and then with 1 μ g/mL of LPS for 5.5 hours. RNA was isolated and reverse transcribed. Gene expression was assayed by quantitative PCR, using *Nono* as the reference gene, and foldchange was calculated vs. the average of the LPS-only condition for each gene. Data are shown as means \pm SE; $n=4$ biological replicates per condition. Data for each gene were analyzed by one-way ANOVA. Significance expressed as * $P<0.05$ and **** $P<0.0001$ vs. LPS-only controls. Results were replicated in three independent experiments.

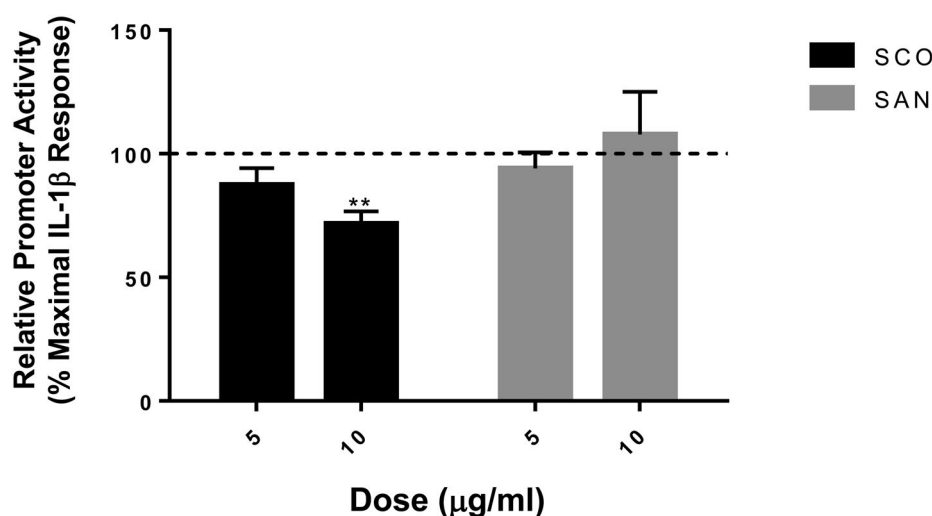


Figure 8 SCO inhibits IL-1 β -stimulated NF- κ B promoter activity in rat insulinoma cells. 832/13 cells were transduced with an adenovirus overexpressing a luciferase reporter construct driven by a promoter containing five copies of a consensus κ B element (5xNF- κ B-Luc). A total of 12 hours after transduction, cells were untreated or treated overnight with 5 and 10 μ g/mL of SCO or an extract of *Artemisia santolinaefolia* (SAN). Cells were then stimulated for 4 hours with 1 ng/mL of IL-1 β . NF- κ B promoter activity was induced 28.6-fold (for the SCO experiment) or 31.1-fold (for the SAN experiment) by IL-1 β treatment (not shown). Promoter activity in cells pretreated with the extracts is expressed as percent of this maximal induction. Data are means \pm SEM of four individual experiments, each performed in triplicate. ** $P<0.01$ vs. control.

In LPS-treated RAW 264.7 macrophages, SCO significantly inhibited *Il6* and *Nos2* expression but had no effect on *Tnfa* expression (Figure 7). This gene-specific response is not entirely unexpected. Toll-like receptor 4 (TLR4) signaling, which mediates the effects of LPS in macrophages, engages two main pathways that utilize distinct subsets of adaptor proteins. The so-called myeloid differentiation primary response 88 (MyD88)–dependent arm results in early phase NF- κ B activation and induction of inflammatory genes, including interleukins. The MyD88–independent arm involves activation of toll/interleukin-1 receptor (TIR) domain-containing adaptor protein inducing interferon beta and interferon regulatory factor 3 (TRIF) and induction of genes such as the type 1 interferons. Although TNF α is an NF- κ B target gene, its regulation by TLR4 signaling is complex (45–47). It has been shown that activation of the NF- κ B–independent TRIF pathway can induce early TNF α production and secretion, which in turn leads to a later-phase secondary autocrine activation of NF- κ B signaling through TNF α receptor 1 (48). In addition, transcriptional activation of NF- κ B target genes is subject to complex regulation by many factors, including chromatin structure and epigenetic status of the target genes, as well as by various posttranslational modifications of NF- κ B subunits and variable subunit combinations of its dimers (46,47,49).

Interestingly, in a similar study in macrophages using a preparation of total flavonoids from *A. scoparia*, LPS-induced levels of *Il6* and *Tnfa* were both inhibited (19). Although the reason for this discrepancy cannot be ascertained, LPS treatment in this study was much longer than ours (20 vs. 5 hours). Under these more chronic conditions, regulation of *Tnfa* expression is more likely to be NF- κ B dependent and thus susceptible to inhibition by SCO. Alternatively, differences in the composition of both extracts could explain these results. Analysis of our SCO extract by chromatography and mass spectrometry has revealed a complex mixture of compounds, many of which are not flavonoids (50). In addition, plants grown in different locations and conditions yield extracts with distinct chemical constituents and bioactivities. Though SCO did not inhibit *Tnfa* gene expression in macrophages (Figure 7), it did attenuate the response to TNF α treatment in adipocytes (Figures 1–2), which could contribute to improving adipose tissue metabolic function in the presence of inflammatory stimuli. Clearly, further studies will be needed to fully elucidate the gene-specific effects of SCO in this context.

Finally, we have shown that SCO reduces NF- κ B–responsive promoter activity in pancreatic β -cells. Recruitment of immune cells and activation of inflammatory pathways are major contributors to the pancreatic β -cell dysfunction that occurs with obesity and insulin resistance, and NF- κ B is known to mediate these processes (36). The finding that SCO attenuates a measure of NF- κ B activation in cultured β -cells (Figure 8) indicates that it can exert anti-inflammatory and metabolically favorable effects beyond adipose tissue.

Our laboratory has studied the effects of SCO on adipocyte differentiation and function, as well as in a DIO mouse model, for several years. We have now shown that SCO significantly impairs inflammatory signaling and transcriptional responses in three cell types that play critical roles in obesity, diabetes, and metabolic syndrome. In addition, we have described effects of SCO on adipocyte levels of DBC1, a protein that has garnered attention in recent years for its involvement in adipogenesis, inflammation, and metabolic dysfunction. We have also discovered that FABP4 secretion, known to be induced by various lipolytic agents,

is stimulated by TNF α , although SCO did not modulate this response. Taken together, our findings reveal that SCO interferes with inflammatory signaling to attenuate responses that promote metabolic dysfunction and we demonstrate, for the first time, that SCO has favorable effects in pancreatic β -cells. These results support further investigation of SCO as a potential nutritional supplement to promote metabolic health in the context of obesity and insulin resistance. **O**

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References

- Engin A. The definition and prevalence of obesity and metabolic syndrome. *Adv Exp Med Biol* 2019;960:1–17.
- Kusminski CM, Bickel PE, Scherer PE. Targeting adipose tissue in the treatment of obesity-associated diabetes. *Nat Rev Drug Discov* 2016;15:639–660.
- Danforth E. Failure of adipocyte differentiation causes type II diabetes mellitus? *Nat Genet* 2000;26:13. doi:10.1038/79111
- Kim J-Y, van de Wall E, Laplante M, et al. Obesity-associated improvements in metabolic profile through expansion of adipose tissue. *J Clin Invest* 2007;117:2621–2637.
- Morigny P, Houssier M, Mouisel E, Langin D. Adipocyte lipolysis and insulin resistance. *Biochimie* 2016;125:259–266.
- Engin A. The pathogenesis of obesity-associated adipose tissue inflammation. *Adv Exp Med Biol* 2017;960:221–245.
- Thomas I, Gregg B. Metformin; a review of its history and future: from lilac to longevity. *Pediatr Diabetes* 2017;18:10–16.
- Richard AJ, Fuller S, Fedorcenco V, et al. Artemisia scoparia enhances adipocyte development and endocrine function in vitro and enhances insulin action in vivo. *PLoS One* 2014;9:e98897. doi:10.1371/journal.pone.0098897
- Richard AJ, Burris TP, Sanchez-Infantes D, Wang Y, Ribnicky DM, Stephens JM. Artemisia extracts activate PPAR γ , promote adipogenesis, and enhance insulin sensitivity in adipose tissue of obese mice. *Nutrition* 2014;30(7–8 suppl):S31–S36.
- Wang ZQ, Zhang XH, Yu Y, et al. Artemisia scoparia extract attenuates non-alcoholic fatty liver disease in diet-induced obesity mice by enhancing hepatic insulin and AMPK signaling independently of FGF21 pathway. *Metabolism* 2013;62:1239–1249.
- Boudreau A, Richard AJ, Burrell JA, et al. An ethanolic extract of Artemisia scoparia inhibits lipolysis in vivo and has antilipolytic effects on murine adipocytes in vitro. *Am J Physiol Endocrinol Metab* 2018;315:E1053–E1061.
- Hussain W, Ullah M, Dastagir G, Badshah L. Quantitative ethnobotanical appraisal of medicinal plants used by inhabitants of lower Kurram, Kurram agency, Pakistan. *Avicenna J Phytomed* 2018;8:313–329.
- Hussain W, Badshah L, Ullah M, Ali M, Ali A, Hussain F. Quantitative study of medicinal plants used by the communities residing in Koh-e-Safaid Range, northern Pakistani-Afghan borders. *J Ethnobiol Ethnomed* 2018;14:30. doi:10.1186/s13002-018-0229-4
- Promyo K, Cho JY, Park KH, Jaiswal L, Park SY, Ham KS. Artemisia scoparia attenuates amyloid β accumulation and tau hyperphosphorylation in spontaneously hypertensive rats. *Food Sci Biotechnol* 2017;26:775–782.
- Sajid M, Rashid Khan MR, Shah NA, et al. Evaluation of Artemisia scoparia for hemostasis promotion activity. *Pak J Pharm Sci* 2017;30:1709–1713.
- Gilani AH, Janbaz KH. Hepatoprotective effects of artemisia scoparia against carbon tetrachloride: an environmental contaminant. *J Pak Med Assoc* 1994;44:65–68.
- Cho J-Y, Park K-H, Hwang D, et al. Antihypertensive effects of artemisia scoparia waldst in spontaneously hypertensive rats and identification of angiotensin I converting enzyme inhibitors. *Molecules* 2015;20:19789–19804.
- Habib M, Waheed I. Evaluation of anti-nociceptive, anti-inflammatory and antipyretic activities of artemisia scoparia hydromethanolic extract. *J Ethnopharmacol* 2013;145:18–24.
- Huang X, Huang H, Ma X, et al. Anti-inflammatory effects and mechanism of the total flavonoids from Artemisia scoparia Waldst. et kit. in vitro and in vivo. *Biomed Pharmacother* 2018;104:390–403.
- Nam S-Y, Han N-R, Rah S-Y, Seo Y, Kim H-M, Jeong H-J. Anti-inflammatory effects of Artemisia scoparia and its active constituent, 3,5-dicaffeoyl-epi-quinic acid against activated mast cells. *Immunopharmacol Immunotoxicol* 2018;40:52–58.
- Cawthorn WP, Sethi JK. TNF- α and adipocyte biology. *FEBS Lett* 2008;582:117–131.
- Weisberg SP, McCann D, Desai M, Rosenbaum M, Leibel RL, Ferrante AW. Obesity is associated with macrophage accumulation in adipose tissue. *J Clin Invest* 2003;112:1796–1808.

23. Escande C, Nin V, Pirtskhalava T, et al. Deleted in breast cancer 1 limits adipose tissue fat accumulation and plays a key role in the development of metabolic syndrome phenotype. *Diabetes* 2015;64:12-22.
24. Moreno-Navarrete JM, Moreno M, Vidal M, et al. Deleted in breast cancer 1 plays a functional role in adipocyte differentiation. *Am J Physiol Endocrinol Metab* 2015;308:E554-E561.
25. Moreno-Navarrete JM, Moreno M, Vidal M, Ortega F, Ricart W, Fernández-Real JM. DBC1 is involved in adipocyte inflammation and is a possible marker of human adipose tissue senescence. *Obesity (Silver Spring)* 2015;23:519-522.
26. Able AA, Richard AJ, Stephens J. Loss of DBC1 (CCAR2) affects TNF α -induced lipolysis and Glut4 gene expression in murine adipocytes. *J Mol Endocrinol* 2018;61:195-205.
27. Mita T, Furuhashi M, Hiramitsu S, et al. FABP4 is secreted from adipocytes by adenyl cyclase-PKA- and guanylyl cyclase-PKG-dependent lipolytic mechanisms. *Obesity (Silver Spring)* 2015;23:359-367.
28. Ertunc ME, Sikkeland J, Fenaroli F, et al. Secretion of fatty acid binding protein aP2 from adipocytes through a nonclassical pathway in response to adipocyte lipase activity. *J Lipid Res* 2015;56:423-434.
29. Hohmeier HE, Mulder H, Chen G, Henkel-Rieger R, Prentki M, Newgard CB. Isolation of INS-1-derived cell lines with robust ATP-sensitive K⁺ channel-dependent and -independent glucose-stimulated insulin secretion. *Diabetes* 2000;49:424-430.
30. Kilroy G, Kirk-Ballard H, Carter LE, Floyd ZE. The ubiquitin ligase Siah2 regulates PPAR γ activity in adipocytes. *Endocrinology* 2012;153:1206-1218.
31. Penforis P, Marette A. Inducible nitric oxide synthase modulates lipolysis in adipocytes. *J Lipid Res* 2005;46:135-142.
32. Li D, Yan Sun W, Fu B, Xu A, Wang Y. Lipocalin-2—The myth of its expression and function. *Basic Clin Pharmacol Toxicol* 2019;bcpt.13332. doi:10.1111/bcpt.13332
33. Zhao P, Elks CM, Stephens JM. The induction of lipocalin-2 protein expression in vivo and in vitro. *J Biol Chem* 2014;289:5960-5969.
34. Burke SJ, Stadler K, Lu D, et al. IL-1 β reciprocally regulates chemokine and insulin secretion in pancreatic β -cells via NF- κ B. *Am J Physiol Endocrinol Metab* 2015;309:E715-E726.
35. Burke SJ, Lu D, Sparer TE, et al. NF- κ B and STAT1 control CXCL1 and CXCL2 gene transcription. *Am J Physiol Endocrinol Metab* 2014;306:E131-E149.
36. Burke S, Collier J. Transcriptional regulation of chemokine genes: a link to pancreatic islet inflammation? *Biomolecules* 2015;5:1020-1034.
37. Burke SJ, Updegraff BL, Bellich RM, et al. Regulation of iNOS gene transcription by IL-1 β and IFN- γ requires a coactivator exchange mechanism. *Mol Endocrinol* 2013;27:1724-1742.
38. Burke SJ, Karlstad MD, Eder AE, et al. Pancreatic β -Cell production of CXCR3 ligands precedes diabetes onset. *BioFactors* 2016;42:703-715.
39. Zhao P, Stephens JM. STAT1, NF- κ B and ERKs play a role in the induction of lipocalin-2 expression in adipocytes. *Mol Metab* 2013;2:161-170.
40. Baker RG, Hayden MS, Ghosh S. NF- κ B, inflammation, and metabolic disease. *Cell Metab* 2011;13:11-22.
41. Chini EN, Chini CCS, Nin V, Escande C. Deleted in breast cancer-1 (DBC-1) in the interface between metabolism, aging and cancer. *Biosci Rep* 2013;33:637-643.
42. Kong S, Dong H, Song J, et al. Deleted in breast cancer 1 suppresses B cell activation through RelB and is regulated by IKK α phosphorylation. *J Immunol* 2015;195:3685-3693.
43. Xu A, Wang Y, Xu JY, et al. Adipocyte fatty acid-binding protein is a plasma biomarker closely associated with obesity and metabolic syndrome. *Clin Chem* 2006;52:405-413.
44. Cao H, Sekiya M, Ertunc ME, et al. Adipocyte lipid chaperone aP2 is a secreted adipokine regulating hepatic glucose production. *Cell Metab* 2013;17:768-778.
45. Doyle SL, O'Neill LAJ. Toll-like receptors: From the discovery of NF κ B to new insights into transcriptional regulations in innate immunity. *Biochem Pharmacol* 2006;72:1102-1113.
46. Vaure C, Liu Y. A comparative review of toll-like receptor 4 expression and functionality in different animal species. *Front Immunol* 2014;5:316. doi:10.3389/fimmu.2014.00316
47. Falvo JV, Tsytysykova AV, Goldfeld AE. Transcriptional control of the TNF gene. *Curr Dir Autoimmun* 2010;11:27-60.
48. Covert MW, Leung TH, Gaston JE, Baltimore D. Achieving stability of lipopolysaccharide-induced NF- κ B activation. *Science* 2005;309:1854-1857.
49. Ahmed A, Williams B, Hannigan G. Transcriptional activation of inflammatory genes: mechanistic insight into selectivity and diversity. *Biomolecules* 2015;5:3087-3111.
50. Boudreau A, Poulev A, Ribnicky DM, et al. Distinct fractions of an artemisia scoparia extract contain compounds with novel adipogenic bioactivity. *Front Nutr* 2019;6:18. doi:10.3389/fnut.2019.00018